CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date January 24, 2003

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

On page 82 and bridging page 83, the last full paragraph:

The gene encoding L chain V region of human antibody 12B5 binding to human MPL was designed by connecting the nucleotide sequence of gene (SEQ ID NO: 67) at the 51'-end to the leader sequence (SEQ ID NO: 68) originated from human antibody gene 3D6 (Nuc. Acid Res. 1990: 18; 4927). In the same way as mentioned above the designed nucleotide sequence was divided into four oligonucleotides having overlapping sequences of 15 bp each (12B5VL-1, 12B5VL-2, 12B5VL-3, 12B5VL-4) and synthesized respectively. 12B5VL-1 (SEQ ID NO: 69) and 12B5VL-3 (SEQ ID NO: 71) had sense sequences, and 12B5VL-2 (SEQ ID NO: 70) and 12B5VL-4 (SEQ ID NO: 72) had antisense sequences, respectively. Each of the synthesized oligonucleotides was assembled by respective complementarity and mixed with the external primer (12B5VL-S and 12B5VL-A) to amplify the full length of the gene. 12B5VL-S ~~(SEQ ID NO: 73)~~ was designed to hybridize to 5'-end of the leader sequence by the forward primer and to have Hind III restriction enzyme recognition site and Kozak sequence. 12B5VL-A ~~(SEQ ID NO: 74)~~ was designed to hybridize to the nucleotide sequence encoding C-terminal of L chain V region by the reverse primer and to have a splice donor sequence and BamHI restriction enzyme recognition site.

On page 83, the first full paragraph:

Performing the PCR as mentioned above, the PCR product was purified by 1.5% low-melting-temperature agarose gel (Sigma), digested by restriction enzymes BamHI and Hind III, and cloned into an expression vector HEF-gκ for human L chain. After determining the DNA sequence the plasmid containing the correct DNA sequence was named HEF-12B5L-gκ. ~~The nucleotide sequence and amino acid sequence of the reconstructed 12B5 L chain V region which were included in plasmid HEF-12B5L-gκ are shown in SEQ ID NO: 75.~~

On page 83 and bridging page 84, the last full paragraph:

The reconstructed 12B5 antibody single chain Fv was designed to be in the order of 12B5VH-linker-12B5VL and to have a FLAG sequence ~~(SEQ ID NO: 76)~~ at C-terminal to facilitate the detection and purification. The reconstructed 12B5 single chain Fv (scl2B5)

was constructed using a linker sequence consisting of 15 amino acids represented by $(Gly_4Ser)_3$.

On page 84, the second full paragraph:

The forward primer 12B5-S (Primer A~~, SEQ ID NO: 77~~) for H chain V region was designed to hybridize to 5'-end of H chain leader sequence and to have EcoRI restriction enzyme recognition site. The reverse primer HuVHJ3 (Primer B~~, SEQ ID NO: 78~~) for H chain V region was designed to hybridize to DNA encoding C-terminal of H chain V region.

On page 84 and bridging page 85, the last full paragraph:

The forward primer RHuJH3 (Primer C~~, SEQ ID NO: 79~~) for the linker was designed to hybridize to DNA encoding the N-terminal of the linker and to overlap DNA encoding the C-terminal of H chain V region. The reverse primer RHuVK1 (Primer D~~, SEQ ID NO: 80~~) for the linker was designed to hybridize to DNA encoding the C-terminal of the linker and overlap DNA encoding the N-terminal of L chain V region.

On page 85, the first full paragraph:

The forward primer HuVK1.2 (Primer E~~, SEQ ID NO: 81~~) for L chain V region was designed to hybridize to DNA encoding the N-terminal of L chain V region. The reverse primer 12B5F-A for L chain V region (Primer F~~, SEQ ID NO: 82~~) was designed to hybridize to DNA encoding C-terminal of L chain V region and to have the sequence encoding FLAG peptide (Hopp, T. P. et al., Bio/Technology, 6, 1204-1210, 1988), two transcription stop codons and NotI restriction enzyme recognition site.

On page 85, the last full paragraph:

In the first PCR step, three reactions A-B, C-D, and E-F were performed, and the three PCR products obtained from the first step PCR were assembled by respective complementarity. After adding primers A and F the full length DNA encoding the reconstructed 12B5 single chain Fv having the linker consisting of 15 amino acids was amplified (the second PCR). In the first step PCR, the plasmid HEF-12B5H-gγl (see Example 7.1) encoding the reconstructed 12B5 H chain V region, pSCFVT7-hM21 (humanized ONS-M21 antibody) (Ohtomo et al., Anticancer Res. 18 (1998), 4311-4316) containing DNA ~~(SEQ ID NO: 83)~~ encoding the linker region consisting of Gly Gly Gly Gly

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser (Huston et al., Proc. Natl. Acad. Sci. USA, 85, 5879-5883, 1988) and the plasmid HEF-12B5L-gκ (see Example 7.2) encoding the reconstructed 12B5 L chain V region were used as templates, respectively.

On page 86 and bridging page 87, the last full paragraph:

The DNA fragments produced by the second PCR were purified using 1.5% low-melting-temperature agarose gel, digested by EcoRI and NotI, and cloned into pCHO1 vector and pCOS1 vector (Japanese Patent Application No. 8-255196). The expression vector pCHO1 was a vector constructed by deleting the antibody gene from DHFR-ΔE-rvH-PM1-f (see W092/19759) by EcoRI and SmaI digestion, and connecting to EcoRI-NotI-BamHI Adaptor (TAKARA SHUZO). After determining the DNA sequence the plasmids containing the DNA fragment encoding the correct amino acid sequence of reconstructed 12B5 single chain Fv were named pCHO-scl2B5 and pCOS-scl2B5. ~~The nucleotide sequence and amino acid sequence of the reconstructed 12B5 single chain Fv included in the plasmids pCHO-scl2B5 and pCOS-scl2B5 are shown in SEQ ID NO: 84.~~